PCT SPECIFICATION

TITLE: **METHOD FOR STABILIZATION OF BIOLOGICAL CULTURES TO ALLOW BIOLOGICAL TREATMENT OF BRINES**

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GOVERNMENT SPONSORSHIP

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RELATED APPLICATIONS

[0002] This application claims provisional priority to United States Provisional Patent Application Serial No. 60/523,637, filed 20 November 2003, incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] The present invention relates to a composition for stabilizing biological cultures in brine solutions under anaerobic/anoxic conditions and to a process for treating brine solutions biologically under anaerobic/anoxic conditions, where the cultures include one or a plurality of microorganisms capable degrading a desired pollutant in a brine solution.

[0004] More particularly, the present invention relates to a composition for stabilizing a biological culture in a brine solution under anaerobic/anoxic conditions, where the composition includes an effective amount of a divalent cation, where the effective amount of the divalent cation is sufficient to produce a divalent/monovalent cation ratio in the brine solution of at least a 0.05 mole/mole or a divalent/monovalent cation ratio greater than or equal to 0.05 mole/mole, where the ratio promotes growth and sustained proliferation of biological microorganisms capable of degrading pollutants or decreasing a concentration of pollutants in the brine solution. The present invention relates to a method using the composition to treat contaminated brine solutions under anaerobic/anoxic conditions. In one preferred embodiment, the stabilized brine solutions are geared to stably grow perchlorate degrading microorganism. In another preferred embodiment, the stabilized brine solutions are geared to stably grow perchlorate and nitrate degrading microorganisms.

2. Description of the Related Art

[0005] Many industrial wastes stream are composed of aqueous salt solutions such as ion-

exchange brines, oilfield production brines, spent caustic solution, and brines produced during chemical processes that contain elevated levels or concentrations of salts such as Na^+. These waste stream may also contain contaminants that would be amenable to biological treatment, microbial treatment, if organisms or microbes could function in high salt waste streams. Some have noted that there is an increasing need for a biological treatment adapted to saline and alkaline environments in industrial wastewater management and that traditional pollutant biodegradation is less efficient or does not function when a salinity of the stream or solution increases above the salinity of seawater.

[0006] Alva and Peyton (2003) examined biological culture growth and phenol degradation at different salt concentrations, but they did not increase the concentration of divalent cations when they increased the Na^+ concentration. Thus, the divalent to monovalent ratio decreased during the study.

[0007] Logan et al. (2001b) screened six sources of inoculum collected from different saltwater environments for perchlorate reduction. After three months incubation, growth was observed in media containing perchlorate and 3% NaCl with inocula from only three sources (seawater, saline lake water and biofilm/sludge). Two of these three (seawater and saline lake water) grew through 3% to 7% salinity in subsequent transfers. They make no mention of increasing the divalent cation concentrations when they increased the Na^+ concentrations in their tests.

[0008] In United States Patent No. 6,077,432 a method for the treatment of wastewater, suspected of being contaminated with perchlorates, nitrates, hydrolysates and other energetic materials is disclosed. The method comprises (a) providing at least one microaerobic reactor containing a mixed bacterial culture capable of reducing perchlorate, nitrate, hydrolysates and other energetic products; (b) feeding contaminated wastewater into the microaerobic reactor; (c) maintaining a microaerobic environment in the microaerobic reactor by at least one method selected from the group consisting of (i) mixing air and nitrogen gas and sparging or purging the reactor with the gas mixture; (ii) using a nitrogen membrane separator to provide a low oxygen-containing nitrogen gas to the reactor for sparging or purging; (iii) adding air to the reactor for sparging or purging as necessary to maintain a target dissolved oxygen concentration or a target oxygen concentration in head space gas present in the reactor; and (iv) adding and/or maintaining oxygenated ions and/or oxygenated molecules; and (d) maintaining suitable nutrient and environmental conditions in the microaerobic reactor so as to cause decontamination of the contaminated wastewater.

[0009] Okeke et al. (2002) obtained cultures that could reduce both perchlorate and nitrate in 0

to 5% NaCl environments, but no effort was made to adjust the divalent cation to monovalent cation ratio.

[0010] Clifford and Liu (1993) developed a sequencing-batch-reactor (SBR) denitrification process to treat and reuse nitrate brine containing 3% NaCl. A pilot study using this ion-exchange process with batch biological denitrification and reuse of the spent brine was conducted successfully in McFarland, California in 1994 where spent brine was denitrified and reused 38 times. (Liu and Clifford, 1996). Compared with a conventional ion-exchange process, brine denitrification and reuse reduced the salt consumption by 50 percent and waste discharge by more than 90 percent.

[0011] Microbial perchlorate reduction under anaerobic conditions has been studied by many researchers. See for example Attaway and Smith, 1993; Herman and Frankenberger, 1999; Logan et al., 2001a; Rikken et al., 1996. Many microorganisms can reduce perchlorate to harmless chloride. Unfortunately, most known perchlorate-reducing microorganisms cannot endure high salinity in the growth media, and usually require less than 2% to 3% NaCl. See for example Coates et al. (2000), Malmqvist et al. (1994), and Michaelidou et al. (2000).

[0012] Several other researchers have conducted salt tolerance tests for the growth of many organisms, but none that changed the divalent cation concentration when the sodium concentration was changed.

[0013] Thus, there is a need in the art for a brine solution capable of stable microbial growth under anaerobic/anoxic conditions and a method to stabilize biological treatment systems in high saline or brine solutions under anaerobic/anoxic conditions.

SUMMARY OF THE INVENTION

[0014] The present invention provides a composition including a brine solution including a pollutant, where the brine solution has an effective divalent to monovalent cation mole ratio and where the effective ratio is sufficient to promote stable microbial proliferation in a brine solution under anaerobic/anoxic conditions, where the microbes are capable of degrading the pollutant under anaerobic/anoxic conditions. Preferred cultures are cultures that are capable of degrading perchlorate and/or nitrate in stabilized brine solutions of this invention.

[0015] The present invention also provides a brine solution including a pollutant and an effective amount of a divalent ratio, where the effective amount is sufficient to adjust a divalent to monovalent cation mole ratio into a range capable of supporting stable microorganism growth and proliferation under anaerobic/anoxic conditions, where the microorganism or microorganisms are capable of reducing a concentration of the pollutant in the brine solution to

a desired level, preferably a level below a set governmental standard or below a detection limit for a governmentally accepted analytical technique. Using the composition of this invention, perchlorates, nitrates, hydrolysates and other energetics can be reduced to a desired low level and preferably below non-detectable concentrations, in a safe and cost effective manner, using readily available non-toxic low cost nutrients. The treatment of this invention results in the degradation of a significantly higher concentrations of perchlorate, nitrate, *etc.* (<1.5 wt %) than was previously possible, especially in brine solution having a salinity greater than 3%. In one preferred embodiment, the salinity ranges from about 3% to about 18%. In another preferred embodiment, the salinity ranges from about 3% to about 15%. In another preferred embodiment, the salinity ranges from about 3% to about 12%. In another preferred embodiment, the salinity ranges from about 3% to about 10%.

[0016] The present invention also provides a brine solution including a pollutant and having an effective divalent to monovalent cation ratio, where the effective ratio is sufficient to stabilize a biological treatment system including at least one microorganism, where the at least one microorganism is capable of reducing a concentration of the pollutant in the brine solution under anaerobic/anoxic conditions, degrading the pollutant in the brine solution or eliminating the pollutant in the brine solution and where a rate of pollutant reduction is similar to (within ±10%) of a rate of pollutant reduction of an equivalently polluted freshwater solution.

[0017] The present invention provides a method including the step of adding an effective amount of a soluble divalent metal complex to a brine solution to form an biologically compatible brine solution, where the effective amount of the complex is sufficient to adjust a divalent to monovalent cation mole ratio to a numeric value greater than or equal to about 0.05 and where the biologically compatible brine solution is capable of supporting and sustaining microbes or microorganisms having pollutant reduction or degradation properties under anaerobic/anoxic conditions.

[0018] The present invention provides a method including the steps of analyzing a brine solution to determine a divalent to monovalent cation mole ratio and adding an effective amount of a soluble divalent metal complex to the brine solution, where the effective amount of the soluble divalent metal complex is sufficient to form an biologically compatible brine solution, where the effective amount of the complex is sufficient to adjust a divalent to monovalent cation mole ratio to a numeric value greater than or equal to about 0.05 and where the biologically compatible brine solution is capable of supporting and sustaining microbes or microorganisms having pollutant reduction or degradation properties under anaerobic/anoxic conditions. By the method

of the present invention, perchlorates, nitrates, hydrolysates and other energetics can be reduced to non-detectable concentrations, in a safe and cost effective manner, using readily available non-toxic low cost nutrients. The method can also be used to degrade other brine solution pollutants by a judicious choice of microbes capable of degrading a given pollutant.

DEFINITIONS

[0019] The term brine solution means any aqueous solution having dissolved therein a sufficient amount of a monovalent alkali metal salt to have a salinity of 3% or more.

[0020] The term microbe means a microorganism capable of degrading a particular pollutant in a stabilized brine solution of this invention, where the exact microorganism will depend on the pollutant to be degraded.

[0021] The term microorganism means a one celled or multi-celled living organism capable of degrading a particular pollutant in a stabilized brine solution of this invention, where the exact microorganism will depend on the pollutant to be degraded.

[0022] Under anaerobic/anoxic conditions mean conditions in which no oxygen or substantially no oxygen is present, by substantially, we mean less than about 500 ppm. As it relates to brine solutions, under anaerobic/anoxic conditions means that the brine solution has no or minimal amount of dissovled oxygen in the solution during the microbial treating step.

DESCRIPTION OF THE DRAWINGS

[0023] The invention can be better understood with reference to the following detailed description together with the appended illustrative drawings in which like elements are numbered the same:

[0024] **Figure 1** depicts a simple schematic of a preferred apparatus of this invention for combined ion-exchange and biological treatment;

[0025] **Figure 2** depicts a plot of data verifying that a biological culture does not degrade perchlorate in the absence of Ca^{2+}, Mg^{2+}, or K^{+} ions in synthetic brine solution containing 60 g/L NaCl;

[0026] **Figure 3** depicts a plot of data showing that when Mg^{2+} is added to an ion-exchange brine solution at different concentrations, the ability for a biological culture to degrade perchlorate rapidly increases with increasing Mg^{2+} concentration;

[0027] **Figure 4** depicts a plot of data showing that when a culture of perchlorate and nitrate reducing organisms were grown in a synthetic medium containing 60 g/L NaCl and 1100 g/L Mg^{2+} a ratio of 0.05 mole Mg^{2+}/mole Na^{+}, perchlorate was degraded to non-detectable levels in 2-8 days, whereas the degradation time was less than one day when the Mg^{2+}/Na^{+} ratio was

increased to 0.1 mole/mole;

[0028] Figure 5 depicts a plot of data showing normalized perchlorate degradation rate demonstrating the effect of Magnesium addition on the degradation of perchlorate from ion-exchange brine; and

[0029] Figure 6 depicts a plot of data demonstrating the effect of magnesium ion concentration on the degradation of perchlorate in an ion-exchange brine.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The inventors have found that a novel composition and method to stabilize biological treatment systems in high saline solution or brine solutions having a high salinity can be constructed where the composition and method permit the sustained growth of microorganisms or microbes capable of reducing pollutant levels in brine solutions under anaerobic/anoxic conditions. The inventors have found that the composition and method are ideally suited for reducing perchlorate ion concentration in ion-exchange brine solutions, again under anaerobic/anoxic conditions. The inventors have also found that through the addition of an effective amount of a divalent cation such as Mg^{2+} or Ca^{2+} to a pollutant contaminated brine solution, microorganisms are capable of growing that degrade pollutants as rapidly and as stably as if the microorganisms were being grown in an equivalently polluted freshwater solution. The inventors are currently unaware of any other process for increasing the salt tolerance of microbial cultures.

[0031] The present invention relates broadly to a brine solution capable of supporting microbial growth under anaerobic/anoxic conditions, where the brine solution has a divalent to monovalent cation mole ratio greater then or equal to 0.05, preferably greater than or equal to 0.1. One preferred embodiment includes a NaCl brine solution having added thereto a sufficient amount of a divalent metal ion, M^{2+} ion, to attain the desired molar ratio. Generally, the divalent to monovalent cation mole ratio is adjusted by adding between about 100 mg/L M^{2+} ions and about 4000 mg/L M^{2+} ions, preferably, between about 500 mg/L M^{2+} ions and about 3000 mg/L M^{2+} ions, particularly, between about 750 mg/L M^{2+} ions and about 2000 mg/L M^{2+} ions, and an optimal level between about 1000 mg/L M^{2+} ions and about 2000 mg/L M^{2+} ions to the solution. Of course, the exact amount of divalent ion to add depends on the initial ratio of the brine solution. For perchlorate ion degradation, the M^{2+} ion is selected from the group consisting of Mg^{2+}, Ca^{2+}, $Sr^{2+}$and mixtures or combinations thereof, where Mg^{2+} is preferred for use in perchlorate contaminated brine solution having high carbonate concentration or in which carbonates are formed. For other pollutants, the M^{2+} ion is selected from the group consisting

of Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, and mixtures or combinations thereof.

[0032] The present invention relates broadly to a method for biologically treating a pollutant contaminated brine solution including the steps of adding an effective amount of a divalent ion source to a brine solution, where the effective amount is sufficient to produce a biologically compatible brine solution capable of supporting and sustaining microbial growth or a biologically stable brine solution. Once the brine solution has been stabilized, a biologically effective amount of a microbial population can be introduced into the solution under anaerobic/anoxic conditions, where the biologically effective amount of the microbial population is sufficient to improve a reduction of pollutant concentrations in the brine solution compared to brine solution in the absence the effective amount of a divalent cation source. In fact, in the presence of the divalent metal cation, the pollutant degradation propensity of the microbials are similar to the pollutant degradation propensity of the microbials in fresh water.

[0033] The present invention relates broadly to a method including the steps of passing a waste water stream through an ion-exchange resin column including an ion-exchange resin capable of extracting perchlorate and/or nitrate ions. After the ion-exchange resin fully loaded with perchlorate an/or nitrate ions or after a sufficient extraction time, the flow of the waste water stream is stopped and a stabilized brine solution of this invention having a divalent to monovalent cation mole ratio of at least 0.05 is passed through the ion-exchange resin to produce a contaminated brine solution, where the stabilized brine solution is capable of supporting and sustaining microbial growth. To the contaminated brine solution is then added a pollutant degrading effective amount of a microbial composition including one microorganism or a plurality of microorganisms each capable of degrading the perchlorate and/or nitrate ions in the stabilized brine solution to form a microbially active brine solution. The microbially active brine solution is agitated under anaerobic/anoxic conditions for at a temperature and for a time sufficient to degrade the perchlorate and/or nitrate concentrations below a desired concentration. After microbial treatment, the microbially active brine solution is filtered to remove the microbial composition. After microbial composition removal, a makeup amount of NaCl is added to the stabilized brine solution where the additional NaCl is sufficient to adjust the salinity of the stabilized brine solution. Optionally, an additional amount of the divalent ion source can be added to the filtered stabilized brine solution, where the amount of additional divalent ion source is sufficient to maintain the ratio of at least 0.05. The stabilized brine solution can then be reused in the perchlorate extraction process. In an analogous fashion, the present method can be adapted for use in treating any type of pollutant contaminated brine solution using a stabilized brine

solution of this invention.

[0034] Unlike prior art aerobic systems that require large amount of specialized nutrients, the present invention operates in the absence of oxygen, i.e., under anaerobic/anoxic conditions, is based on adjusting the divalent to monovalent cation mole ratio of the brine solutions to promote microbial growth and proliferation and requires only the addition of acetate as a nutrient so that the degrading brine solutions of this invention are simpler, easier to maintain and more stable the prior art brine solutions used to degrade pollutants.

Suitable Material for Use in the Present Invention

[0035] suitable divalent ion source for use in this invention includes, without limitation, any soluble divalent metal salt, where the counterion does not adversely after the culture. Exemplary examples of the divalent metal salts include, without limitation, divalent metal chlorides, divalent metal bromides, or mixtures or combinations thereof. The preferred salts are chloride salts.

[0036] suitable divalent ion for use in this invention include, without limitation, magnesium, calcium, strontium, other similar divalent metal cations capable of promoting microbial growth in brine solutions or mixtures or combinations thereof.

[0037] Suitable microbials or microorganisms for use in this invention include, without limitation, bacteria from capable of growing in the stabilized brine solutions of this invention and capable of degrading the pollutant of interest.

[0038] suitable pollutants which can be degraded using the compositions and methods of this invention include, without limitation, inorganic pollutants, organic pollutants, or mixtures or combinations thereof. Exemplary inorganic pollutants include, without limitation, perchlorates, nitrates, nitrites, or mixture or combinations thereof. Exemplary organic pollutants include, without limitation, phenols, PCBs, chlorinated solvents, solvents, sewage, industrial wastes, oils, sludge, other chemical pollutants or mixtures or combination thereof.

[0039] Suitable solid medium for supporting microbial growth include, without limitation, diatomaceous earth, activated carbon, sand, ion-exchange resin, or mixtures or combinations thereof.

[0040] Suitable reactors for use in the treating step of this invention include, without limitation, a plug flow, dispersed plug flow, or continuously stirred tank reactor, or as a packed, expanded, or fluidized bed column.

EXPERIMENTAL SECTION

[0041] The examples below illustrate that adjusting a divalent/monovalent cation mole ratio in waste brine solutions results in an improved, reusable and safe disposal system or treatment

system for treating polluted brine solutions, especially brine solutions including pollutants such as perchlorate and nitrate ions.

General Background of Perchlorate-Contaminated Brine Solutions

[0042] Perchlorate (ClO_4^-) is a contaminant found in groundwater that can be removed by an ion-exchange process using an ion-exchange resin. During the process, the resins are regenerated resulting in the formation of brine solutions contaminated with perchlorate. These brine solutions are largely defined by a concentration of NaCl in the brine solution used to regenerate the resin. Typically, the NaCl concentration ranges from as low as about 30 g/L NaCl (a 3% saline solution or a 0.5 M NaCl solution) to as high as about 90 g/L (a 9% saline solution or a 1.5 M NaCl solution). These brine solutions represent waste streams requiring disposal. Generally, the higher the NaCl concentration of the regenerant brine solution, the smaller a volume of the perchlorate-contaminated brine solution generated. These brine solutions can also contain nitrates.

[0043] Although these brine solutions can now be disposed of directly into the environment, the EPA is planning to enact legislation that will forbid the disposal of perchlorate-contaminated brine solutions directly into the environment. The ability to remove nitrate and perchlorate from such brine solutions will allow brine solution disposal, and more importantly, will allow the treated brine solutions to be continually recycled in an ion-exchange process. The ability to recycle the treated brine solution will result in a conservation of salt and decrease disposal costs.

[0044] Typical water treated in ion-exchange processes includes about 50 to about 100 µg/L perchlorate and between about 1 to about 20 mg/L nitrate-N. After treating, a brine solution is produced including between about 2.5 and about 10 mg/L perchlorate and between about 150 and about 500 mg/L nitrate-N. For more details on typical waste water stream the reader is direct to Tripp and Clifford, (2000) and Najm et. al. (1999).

[0045] Coppola (1999) Coppola et al. (2000) reported that HAP-1 or a strain of *Wolinella succinogenes* could reduce perchlorate in brine solution having 2% to 3% NaCl in the presence of high concentrations of nitrate, sulfate, ammonia and chlorate. The culture could not grow at higher salt concentrations and required strict maintenance of microaerophillic conditions and the addition of rich nutrients.

[0046] Okeke et al. (2002) obtained cultures that could reduce both perchlorate and nitrate in solutions having 0 to 5% NaCl. A *Citrobacter* isolate was reported to provide the fastest nitrate and perchlorate removal in conjunction with their Perclace™ culture, removing 46.4% of the perchlorate fed to it in one week. However, because typical ion-exchange columns treating

perchlorate and nitrate will be exhausted in less than 24 hours, a culture should be able to remove nitrate and perchlorate in less than 24 hours to avoid having to store large volumes of brine for remediation.

Example

[0047] This examples illustrates the biological treatment of perchlorate and nitrate contaminated ion-exchange brines.

[0048] One preferred method of this invention includes the step of using an ion-exchange resin to remove perchlorate from a polluted water. Once the resin is no longer capable of removing perchlorate, the resin is regenerated using a brine solution to produce a perchlorate contaminated brine solution. To the perchlorated contaminated brine solution is added an effective amount of a divalent cation precursor sufficient to adjust a divalent to monovalent cation mole ratio in the perchlorate contaminated brine solution to a numeric value greater than or equal to about 0.05. After adjusting the ratio in the brine solution, a treating effective amount of a biological treating composition is added to the brine solution and the solution is agitated for a time and at a temperature sufficient to reduce the perchlorate and/or nitrate concentration to or below a desired low level. The biological treating composition includes at least one microorganism capable of degrading perchlorate ions to chloride ions.

[0049] Referring now to **Figure 1**, a block diagram of a preferred embodiment of an apparatus for implementing a method of this invention, generally **100**, is shown to include an ion-exchange column **102** filled an ion-exchange resin **104** and having a contaminated waste water input **106**, a brine solution input **108**, a treated water output **110** and a brine solution output **112**. The waste water input **106** is connected to a source of waste water (not shown). Waste water containing ion-exchangeable contaminants including perchlorate and nitrate ions flows from the source through the waste water input **106** and passes through the ion-exchange column **102** and exchanges its ion contaminants to the resin **104** until the ion-exchange resin **104** is no longer capable of exchanging the contaminant ions or for a set period of time. After the specified time or after full exchange of the resin **104**, the waste water input **106** is closed by a valve or other similar shut off device (not shown) and the brine solution input **108** is opened by a valve or other similar shut off device (not shown) is connected to a brine solution treatment reactor **114**. The reactor **114** includes a brine solution input **115** a microbial nutrient input **116** connected to a microbial nutrient input source (not shown). The reactor **114** can also include a microbial input for adding microbes to the reactor **114** to maintain an effective concentration of viable microbes in the reactor **114**. The reactor **114** also includes a crude treated brine solution output **118**

connected to a filter tank **120** including a filter **122**, where the microbes in the crude treated brine solution are removed by the filter **122**. The filtered treated brine solution flows out of the filter tank **120** via a filtered, treated brine solution output **124** connected to a brine holding tank **126**. The holding tank **126** includes a make up NaCl input **128** connected to a NaCl source (not shown). The holding tank **126** can also include a divalent cation precursor input connected to a source (not shown). In a batch mode, the process would run waster water through the resin column until the resin was exhausted.

[0050] The waster water feed would then be shut off and the column regenerated. The resulting brine solution is then treated by microbes to remove the pollutants in the brine. In a continuous mode, the process would include two or more resin columns. One column used to process the waste water while the other is being regenerated. A brine solution is continuously being treated to remove the ion exchanged contaminants, filtered, stored, adjusted with additional NaCl and divalent cation precursor, and recirculated to the regenerating column. In the continuous mode, not only is nutrients added to the treating reactor, but microbes are also added to maintain a treating level of microbes in the reactor. The entire process is sealed as much as possible to prevent air and the biological reactor is sparged with nitrogen gas to maintain anoxic/anaerobic conditions. The Mg^{2+} is maintained at the optimal ratio to Na^{+} for the best culture stability in the specific brine solutions by addition to the spent brine storage tank. Makeup sodium chloride is added in the sweet brine storage tank. Alterations to the proposed diagram include the use of continuous culture to replace the batch culture. A media filter immediately follows the biological treatment unit to prevent any organisms that did not settle in the reactor from coming in contact with the resin bed.

[0051] The initial attempts to develop a biological culture that could treat brine solutions having a high salt content from a sewage sludge inoculum were unsuccessful. Cultures were then obtained that could degrade perchlorate and/or nitrate, which are typical pollutants in waste water brine solutions, but these microbes could not be adapted to any more than 15 g/L NaCl. When marine sediments were used as an inoculum, the inventors were able to develop two cultures that could degrade perchlorate and nitrate in synthetic media containing 30 or 60 g/L NaCl. These cultures degraded perchlorate and nitrate simultaneously, and require the complete absence of oxygen from the headspace and the media. That is, the cultures, which includes a collection of microorganisms, some identified and some not yet identified, degrade these pollutants under strict anaerobic/anoxic conditions.

Culture Development

[0052] A culture developed from marine sediment that was capable of degrading perchlorate and/or nitrate in 30 g/L NaCl synthetic media in the first feedings of ion-exchange brine became unstable after removal of biomass to perform subsequent experiments. This culture did not maintain or increase biomass as most biological cultures do. The culture developed from marine sediment and raised in 60 g/L NaCl synthetic medium could not degrade perchlorate at all in a 60 g/L NaCl ion-exchange brine solution.

Materials and Methods for Microbe Selection

[0053] In this research, two approaches were taken to the development of cultures capable of reducing perchlorate and nitrate in solutions of 30 or 60 g/L NaCl. The first was to enrich a population of perchlorate- and/or nitrate-reducing organisms from sewage, and then acclimate these to increasing salt concentrations as was done by Clifford and Liu [7]. The second was to screen six marine sediments for their use as inoculum for developing salt-tolerant perchlorate-reducing cultures. **Table 1** presents a summary of the experimental and culture conditions tested in this research.

Perchlorate- and Nitrate-reducing Culture Development from Marine Inocula

Screening Tests in 3% and 6% Nacl

[0054] Six anaerobic near-shore marine sediments were sampled and shipped in well sealed white-plastic buckets. They were kept refrigerated (4 °C) and well sealed between uses.

Table 1
Summary of Experimental Conditions

ID	Media description[a]	Inoculum	Variables	NaCl (mg/L)	Mode
I Sewage 1	1	Sewage	Stepwise adaptation to NaCl	8–20 g/L NaCl	Spike and SBR
I Sewage 2	2	Sewage	Stepwise adaptation to NaCl	8–20 g/L NaCl	Spike and SBR
I Sewage 3	3	Sewage	Stepwise adaptation to NaCl	8–20 g/L NaCl	Spike and SBR
IIa Screening 1	4, 5, 6, 7	6 Marine sediments	Inoculum source, nitrate, yeast extract	30 g/L	Single batch
IIa Screening 2	8, 9	6 Marine sediments	Inoculum source, nitrate, yeast extract	60 g/L	Single batch
IIb Large Culture 1	6 then 4	Freeport Sediment	Perchlorate and nitrate then perchlorate alone	30 g/L	Spike and SBR
IIc Ingredients	4a, 4b, 4c	Marine Culture 1	Fresh sediment, S^{-2}, trace metals, phosphate	30 g/L	Single batch
IId, e Large Culture	4c 2	Culture fed medium 4c in IIc	Perchlorate then nitrate	30 g/L	Spike and SBR

ID	Media description[a]	Inoculum	Variables	NaCl (mg/L)	Mode
IIf Marine Culture 3	4c with 60 g/L NaCl	Freeport culture from screening exp 2	Perchlorate	60 g/L	Spike and SBR

[a]See text for media ingredients corresponding with each medium number.

[0055] Six different synthetic media were used to test the ability of the marine sediments to reduce perchlorate in the presence of 30 and 60 g/L NaCl. All six media contained the following basal ingredients per liter of deionized water; 11 g $MgCl_2 \cdot 6H_2O$, 1.4g $CaCl_2 \cdot 2H_2O$, 0.2g $NaHCO_3$, 0.72g KCl to represent the major components of seawater and 0.59g NH_4ClO_4, 10g $NaCH_3COO \cdot 3H_2O$ to supply perchlorate and an electron donor. Sulfate was omitted from the media to prevent the growth of sulfate-reducing bacteria, which could compete with perchlorate-reducing bacteria for the electron donor. The inocula contained large amounts of reduced sulfide, which was expected to act as a sulfur source for organism growth. Four media containing the basal ingredients specified above were prepared in 30 g/L NaCl: Medium 4 contained no additions; Medium 5 contained 1 g/L yeast extract; Medium 6 contained 0.685 g/L $NaNO_3$; Medium 7 contained 1 g/L yeast extract plus 0.685 g/L $NaNO_3$. Two media containing the basal ingredients plus the following additions were made up at 60 g/L NaCl: Medium 8 contained 1 g/L yeast extract, Medium 9 contained 0.685 g/L $NaNO_3$ and 1 g/L yeast extract.

[0056] The screening tests were performed by adding 3 g of each anaerobic marine sediment to 100 mL of each medium in a 125-mL serum bottle. The dissolved oxygen in the medium was not removed, however, the headspace of the serum bottle was purged with nitrogen gas for 3 min. The serum bottles were crimp-sealed with butyl-rubber stoppers and mixed on a rotary shaker and incubated at 3072'C for at least one month. Perchlorate and nitrate (when present) were measured as described below.

First Large Culture Development in 3% Nacl

[0057] A fresh sample of 3% (w/v) of Freeport #1 sediment was added to 1.5 L Medium 6 in a 2-L glass bottle reactor with a gas-collection device. The reactor was incubated at 30±2°C and shaken at a rate of 150 rpm. After the initial nitrate and perchlorate in the reactor were removed, 100 mg/L perchlorate was spiked into the reactor. Nitrate was not included in subsequent spikes or feeds until a stable perchlorate reducing culture was developed. This spike-feed procedure was continued until a reproducible perchlorate reduction rate was obtained. Then the feed protocol was switched from a spiked batch reactor mode to a sequencing-batchreactor (SBR) mode using a 30% replacement.

Batch Medium Ingredient Experiments in 3% NaCl

[0058] A 10-mL inoculum from the 1.5-L perchloratereducing culture, which had lost activity due to SBR operation, was placed into 90 mL of medium 4a, 4b, or 4c prepared and dispensed into 125-mL serum bottles using strict anaerobic technique. Medium 4a 4a was prepared by adding 67 mM $Na_2S \cdot 9H_2O$, to Medium 4. Medium 4b was prepared by adding 0.1 mL trace metal solution and 0.1 mL 50 g/L KH_2PO_4 to Medium 4. Medium 4c was prepared by adding 0.5 mL 67 mM $Na_2S \cdot 9H_2O$, 0.1 mL trace metal solution, and 0.1 mL 50 g/L KH_2PO_4. The trace metal solution consisted of 10 g ammonium molybdate, 0.1 g zinc sulfate, 0.3 g boric acid, 1.5 g ferrous chloride, 10 g cobalt chloride, 0.03 g magnesium chloride, 0.03 g nickel chloride, and 0.1 g aluminum potassium sulfate per liter of water.

Second Large Culture Development in 3% NaCl

[0059] A second 1.5-L culture was enriched using Medium 4c by increasing the volume of the 90-mL culture from the nutrient test that had received Medium 4c by addition of fresh medium in 500 mL batches each time perchlorate was reduced to nondetect levels. The culture was maintained by spiking 100 mg/L perchlorate every three days. After every five feeds, 1 g/L sodium acetate$\cdot 3H_2O$ was spiked into the reactor as well. For several spike-feed cycles, samples were taken every two hours to measure the perchlorate concentration in the reactor.

Demonstration of the Effect of Nitrate in 3% NaCl

[0060] On the 8th feed of the second large culture, 500 mg/L nitrate-N was spiked with 100 mg/L perchlorate. Sodium acetate (3 g/L) was added as the electron donor for both perchlorate and nitrate reduction. Samples were again taken every 2 h, and the nitrogen gas produced in the reactor was measured in the gas collector. Both nitrate and perchlorate were spiked into the reactor for another two feeds when the perchlorate and nitrate in the current allotment of feed was reduced.

Perchlorate Reduction at 6% NaCl

[0061] Inocula of 10 mL of the Freeport #1 culture that reduced perchlorate at 60 g/L NaCl in the screening experiments were transferred to serum bottles containing 90 mL of Medium 4c adjusted to 60 g/L NaCl. After all of the perchlorate in the medium was removed, 100 mg/L perchlorate was spiked into the culture again. This feed procedure was continued 5–6 times to allow more cell mass to grow. Then 10 mL of the culture was transferred again to 90 mL fresh medium and spiked several times. Samples were taken to test perchlorate reduction by the culture at 60 g/L NaCl.

Analytical Methods

[0062] Samples of 1 mL (serum bottle tests) or 5 mL (1.5-L culture tests) were taken using

nitrogen-flushed sterile syringes and filtered through 0.20 mm sterile syringe filters immediately after sampling, and kept in a refrigerator at 4°C if not analyzed that day.

Nitrate, sulfate, chlorate and perchlorate were measured using a Dionex DX-800 ion chromatograph configured with a GS50 gradient pump, CD25 conductivity detector, an ASRS-ULTRA suppressor, and an AS40 automated sampler. The suppressor was set at 300 mA. Separation was obtained using a Dionex

[0063] IonPac AS16 anion analytical column (4 mm x 250 mm) mm) and an AS16 guard column (4 mm x 50 mm). A 225-mL sample loop was used to measure perchlorate concentration higher than 1 mg/L. The sample loop was switched to 1000 mL to measure lower perchlorate concentrations. The detection limit for perchlorate was 5 ppb in de-ionized water and 500 ppb in the presence of X8 g/L NaCl concentration. A gradient eluent was delivered in order to separate all peaks: Initially, a flow of 5 mM KOH was maintained for 2 min at a flow rate of 1.0 mL/min. The eluent KOH composition was changed to 10 mM in a linear gradient from 2 min to 14 min with the flow rate unchanged. A linear gradient was then used to change the eluent composition to 55 mM KOH from 14 min to 20 min while the flow rate was increased to 1.5 mL/min at 20 min. These conditions were held constant from 20 to 27 min. All water used was de-ionized, reagent grade with 18 MO cm resistivity.

[0064] Nitrite was analyzed by absorbance using the method described in *Methods of Seawater Analysis* [26] because it could not be resolved from the chloride peak during IC analysis. The absorbance was measured in 1-cm cuvettes at 540 nm with Lambda 3B UV/VIS spectrophotometer, Perkin-Elmer Corporation.

Culture Development from Activated Sludge

[0065] The three cultures developed from activated sludge were fed acetate as the electron donor and (1) nitrate and perchlorate, or (2) perchlorate only, or (3) nitrate only as the added electron acceptors at an initial NaCl concentration of 8 g/L.

[0066] All three cultures were able to adapt quickly to the removal of perchlorate and nitrate from the media when the NaCl concentration was 8 g/L. The two cultures fed perchlorate only could not tolerate more than 15 g/L NaCl in the media. The culture fed with perchlorate and nitrate never showed recovery from any step increase in salt concentration. These results suggest that the presence of nitrate may have some negative effect on perchlorate reduction at higher salt concentrations. Because the culture fed with perchlorate alone did not acclimate to more than 15 g/L NaCl, there must be other physiological problems as well.

[0067] The fact that neither culture was able to acclimate to the targeted 30 g/L NaCl

concentration whether or not nitrate was present, demonstrates that the sewagesludge-acclimation approach was not a successful strategy to obtain a culture capable of reducing perchorate and nitrate at 30 g/L NaCl. The control culture that was fed only nitrate was able to adapt to 30 g/L NaCl with no apparent problems.

Culture Development from Marine Sediment

Screening Experiments in 3% and 6% NaCl

[0068] A 30-day sample of six marine sediments incubated in synthetic media with 30 or 60 g/L NaCl revealed that the organisms in only three of the sediments—Freeport #1, Fourchon #1 sand Fourchon #3—were capable of reducing perchlorate. All l six sediments reduced at least 98% of the nitrate in all of the media having nitrate (results not shown).

[0069] In the presence of 60 g/L NaCl, no perchlorate reduction was observed by the 30-day sampling period while at least 98% of the nitrate in all of the media that contained nitrate was reduced.By the 45-day sample, the Freeport # 1, Fourchon #1 and Fourchon #3 sediments showed perchlorate reduction.Again, the other three sediments did not show much perchlorate reduction.

First Large Culture Development in 3% Nacl

[0070] The Freeport #1 sediment was selected as the most consistent inoculum and Medium 4 containing perchlorate and nitrate at 30 g/L NaCl was selected as the growth medium to enrich a larger-scale perchlorate reducing culture. This 1.5-L Freeport ##1 culture experienced a 28-day lag period, but was then able to reduce 510 mg/L perchlorate to 4.93 mg/L within 56 days. Nitrate was reduced within the first week of incubation. Thereafter, along with each spike feed of 100 mg/L perchlorate, the perchlorate reduction rate increased, and an increase of the biomass was observed in the reactor. After 3–4 perchlorate spikes, the culture could remove 90% of perchlorate fed in the medium within 30 h.

[0071] In order to simulate the ion-exchange brine reuse process the culture was then operated under SBR mode. This dramatically decreased perchlorate reduction. It took more than six days to reduce the same amount of perchlorate for the first feed in the SBR mode, and more than nine days for the second feed using SBR conditions.This suggested that some ingredient in the initial mud inoculum that was important for perchlorate reduction by the culture was depleted during medium replacement.

Batch Medium Ingredient Experiments in 3% NaCl

[0072] The original Freeport 1 marine sediment was rich, black, and very anaerobic. To determine if there were abiotic factors present in the mud that enabled the culture to reduce perchlorate rapidly, fresh, autoclaved Freeport marine sediment was added to duplicate transfers

of the ineffective large culture to determine if this could return the culture to a rapid perchlorate reduction rate. Adding the autoclaved sediment had a beneficial effect.The culture containing sediment-amended medium had less perchlorate remaining after a five-day incubation period than the controls.This trend was again observed after a second spike of perchlorate into the cultures (not shown).

[0073] The most obvious abiotic factors in the sediment that could be beneficial to the culture were sulfide or other mineral nutrients.To determine which components might be responsible for the beneficial effect, Na_2S and trace minerals were added to the culture.The addition of phosphate as a traditional biological nutrient was also examined.The addition of Na 2S, trace metals and phosphate together caused the most beneficial effect. The addition of Na2S alone somewhat improved perchlorate reduction, whereas trace metals and phosphate only had no beneficial effect (results not shown). Na2S provides sulfur for microbial growth, scavenges oxygen, and reduces the redox potential in the culture. Lower redox potential is helpful to anaerobic perchlorate reduction.Trace metals and phosphate are important to the bacteria's growth and metabolism, especially for bacteria growing in strict environments (anaerobic and high saline).From these results, this marine culture needs both low redox potential and trace metals to reduce perchlorate.

Second Large Culture Development in 3% NaCl

[0074] The culture growing in trace metal-, phosphate- and Na2S- amended Medium 4c was used to create another 1.5 L culture. After several spike feeds of B100 mg/L perchlorate, this culture was capable of removing 70– 100 mg/L perchlorate within 8 h. After r 48 daily

[0075] SBR feedings of Medium 4c from this point, samples were collected every two hours and analyzed for perchlorate during one react phase. The results showed that the culture performance was stable. Thisculture continued to reduce its allotment of perchlorate in each daily feed or SBR operation for two months.

Demonstration of the Effect of Nitrate in 3% NaCl

[0076] The ability of the culture that could degrade perchlorate successfully in 30 g/L NaCl to reduce perchlorate in the presence of nitrate was also tested. The addition of 9 x as much nitrate (molar basis) as perchlorate did not effect the perchlorate reduction by the culture. Perchlorate was reduced within 6 hour with or without the presence of nitrate. The perchlorate reduction curves were modeled with first order kinetics and the k-values were 0.627/h and 0.514/ hour. Nitrate (9 mM) was also reduced within 10 h.

[0077] The culture adapted to denitrification very quickly by the third spike feed, when the 92 mg/L perchlorate and 539 mg/L nitrate-N were both reduced (98%) within 5 h. At least 153 mL nitrogen gas was collected, which is comparable to the theoretical gas production (147– 155 mL assuming 1 mol NO_3 was converted to 0.45– 0.48 mol nitrogen gas, 30°C, one atm. total pressure, and water-saturated air). This suggests that this marine culture can denitrify at a rapid rate along with the reduction of perchlorate.

[0078] A microscopic examination of a Gram-stained sample of the culture revealed that this was not a pure culture but the majority of the organisms present in the culture were Gram-negative, slightly curved rods.

Perchlorate Reduction at 60 g/L NaCl

[0079] Although 30 g/L NaCl can be used to regenerate the perchlorate-spent resin, the preferred concentration of NaCl in the ion-exchange brine is 60 g/L (6%) or higher. Initial batch screening tests provided a culture that was initially capable of reducing perchlorate in a medium that contained 60 g/L NaCl within 45 days, but lost the capability in the subsequent transfer to fresh medium with 60 g/L NaCl. Once it was learned that the 30 g/L culture required sulfide, trace metals and phosphate, these ingredients were added to revive the culture in the 60 g/L medium. After 1 or 2 transfers to fresh Medium 4c adjusted to 60 g/L NaCl and several spike feeds of 100 mg/L perchlorate, a stable culture capable of reducing perchlorate within 1 day at 60 g/L NaCl was obtained. The culture was capable of removing more than 90% of 80–100 mg/L perchlorate within at most 29 hour. The data from the curves presented in Fig. 5 were modeled using zero-order kinetics. The average perchlorate degradation rate was 3.61 mg/L h. The fit to a zero-order curve suggests that there are low numbers of perchlorate-degrading

microbes present in this culture so the degradation rate is saturated even at low perchlorate concentrations.

[0080] The pathway of perchlorate degradation involves the sequential reduction of perchlorate to chlorate, chlorite, and finally, chloride.The he analytical method used allowed the detection and quantification of perchlorate, and chlorate, but not chlorite.The chloride produced from the reduction of perchlorate could not be quantified because of high background of NaCl (3–6%) in the media.For the culture enriched from the Freeport #1 sediment, chlorate was observed only transiently in early enrichment cultures, but was never observed in mature cultures.The completion of the respiration of perchlorate can be inferred by a change in redox potential indicated by the color change of resazurin due to O_2 produced in the final reaction. This was observed, again, in enrichment cultures, but rarely in the mature cultures.This does not mean that complete metabolism was not achieved but only that the O_2 was removed as fast as it was produced.

[0081] An electron balance was conducted for electron use by the perchlorate-reducing culture enriched from Freeport #1 sediment.In five spike feed cycles, 40 meq of perchlorate (ClO_4^- to Cl^-) were fed and 59 meq of acetate (CH_3COO^- to CO_2) were used.The electron equivalence of acetate is higher than the equivalence of perchlorate, which supports a total reduction of perchlorate to chloride and indicates that acetate was also used for biomass generation.

[0082] Two cultures capable of degrading perchlorate and nitrate in high salt solutions were developed from marine inoculum.One culture is capable of reducing up to 100 mg/L perchlorate and 500 mg/L nitrate-N within 5 h in the presence of 30 g/L NaCl.The other is capable of reducing 100 mg/L perchlorate in the presence of 60 g/L NaCl within 24 h.The growth conditions to maintain these cultures in a healthy state require the maintenance of strictly anaerobic conditions and the addition of trace metals, Na_2S and phosphate.

Inocula

[0083] Mixed cultures capable of perchlorate and nitrate reduction in the presence of 3% and 6% NaCl synthetic medium were used as inocula in this study. The anaerobic cultures were enriched from marine mud and have been fed with synthetic medium as shown in Table 2 in the lab for an extended period of time to establish proven perchlorate and nitrate degrading capacity.

TABLE 2
Comparison of Ingredients in Synthetic Medium and 2 Ion-exchange Brines

Component	3% Synthetic Medium	3% Spent Pilot Plant Brine*	Ion-Exchange regenerant Brine
pH	7	9.1	8.1
NaCl	30 g/L	30 g/L	53 g/L
Mg^{2+}	1300 mg/L	6.5 mg/L	**
Ca^{2+}	380 mg/L	24.5 mg/L	0
K^+	380 mg/L	None	**
NH_4^+	Present	2 mg/L	**
NO_3^-	None	510 mg/L	2000 mg/L
SO_4^{2-}	None	800 mg/L	2810 mg/L
ClO_4^-	400 µg/L	1400 µg/L	4300 µg/L
HCO_3^-	145 mg/L	00 mg/L	11000 mg/L
$H_2PO_4^-$	35.6 mg/L	None	**
$Na_2S{\cdot}9H_2O$	80 mg/L	None added	None added
Trace Metal	Present	None added	None added

*Estimated based on MWH analysis and measurements of components of ion-exchange spent brine.
**Not analyzed for in the ion-exchange regenerant brine.

[0084] The cultures were maintained in six, 1.5-L sealed glass bottle reactors as sequencing batch reactors by weekly settling, decanting 50-60% of the supernatant (spent medium) with fresh synthetic media typically once a week. Between the replacements, perchlorate stock solution (100 g/L) was spiked in the cultures to a final perchlorate concentration of about 100 mg/L, daily. Acetate served as the sole electron donor. Prior to each experiment, volatile suspended solids (VSS) concentration was measured for the Parent culture so that VSS concentration present in the subcultures could be estimated.

[0085] The inoculum for the pilot plant was prepared by taking 500 mL of the 3% synthetic medium culture and increasing the volume in several steps (including some spikes of perchlorate to high levels to increase biomass) until the culture was 20 gallons.

Pilot Plant Operation and Brine Generation

[0086] A spent brine solution from the ion-exchange process, ion-exchange brine solution, was collected to be representative of an average of a full range of brine solution quality encountered throughout a cycle of ion-exchange column regeneration. The sample was transported in a headspace free 15-gallon container and stored at 4°C until use in individual experiments. Chemical analysis and microbial characterization tests were conducted on the samples of brine as received. Table 2 lists the major cations present in the brine after dilution to 3% NaCl with deionized water.

Experimental Conditions

[0087] Initial laboratory studies were performed by omitting specific medium ingredients from

the synthetic medium (see Table 2). These experiments suggested that the divalent cations (Ca^{2+}, Mg^{2+}) and the monovalent cation (K^+) were important ingredients in the synthetic medium. When these items were omitted the perchlorate reduction rate was reduced.

[0088] To confirm that the addition of these minerals to the brine would improve the stability of perchlorate degradation in brine solution by the culture three experiments were performed. Two using brine solution generated using the pilot plant as described above (and conducted simultaneously in the lab and pilot plant) and one using an ion-exchange regenerant brine solution.

Bench Scale Pilot Plant Brine Treatment

[0089] Since initial results suggested that the culture could not directly treat 6% pilot plant brine solution, the brine collected from the pilot plant for treatment was diluted to 3% NaCl by the addition of an equal amount of deionized water which had been boiled and cooled under a flush of nitrogen gas. Ambient oxygen was purged from the brine by bubbling with oxygen-free nitrogen gas for approximately 1 hour and 0.3 mg/L resazurin was added as a redox indicator.

[0090] Inoculum from the 3% NaCl parent culture was prepared for use (*i.e.*, the residual nutrient components from the parent culture were removed) by centrifuging the culture (1500 rpm, at 4°C) for approximately 12-25 minutes. After centrifuging, the supernatant was decanted and resuspended in 3% NaCl solution. This procedure was repeated twice. The final harvested cell pellet was re-suspended in 3% NaCl solution and 2 mL of this suspension was added to about 100 mL of amended brine solution in a 150 mL serum bottle that had been flushed with N_2 gas for at least five minutes and then sealed with butyl rubber stoppers and aluminum crimp seals. The results from Mg^{2+} and Ca^{2+} measurements showed that insignificant amounts of the cations were carried over with the inoculum. The cultures were spiked with about 100 mg/L acetate and the appropriate amounts of concentrated metal ions (individually or all together) to adjust the concentrations of Mg^{2+} to 130 mg/L, Ca^{2+} to 40 mg/L, or K^+ to 40 mg/L as their chloride salts. The initial pH of the brine was adjusted to 7.5 during the initial transfer and no pH adjustment was done during the subsequent SBR feeds. The cultures were incubated while shaking at about 120 rpm at room temperature.

[0091] The performance of the resulting subcultures, namely, Mg, Ca, K, and (Mg+Ca+K) (in triplicate), was compared with the performance of triplicate subcultures fed brine with no cation amendment. At the end of the first incubation period (while the transfer removed its perchlorate and nitrate), there was approximately 40 mL of culture left in each serum bottle due to frequent sampling. The first SBR feed was accomplished by adding 60 mL of 3% diluted spent brine, and

subsequent feeds were accomplished using true SBR procedures. The cations and acetate were spiked directly into the cultures to the levels described above at the beginning of each feed cycle. The magnesium and calcium concentrations were measured at the beginning and end of the experiment by flame atomic absorption spectrometry.

Biological Treatment of Brine at the Pilot Plant

Effect of Magnesium on Treatment of ion-exchange brine solution

[0092] To evaluate the impact of magnesium addition on the biological treatment of ion-exchange brine solution, two 300 mL cultures were developed from a combination of 3 and 6% NaCl parent cultures and fed to an ion-exchange brine solution that had been purged with nitrogen to scrub oxygen, and amended 22 g/L $MgCl_2 \cdot 6H_2O$ (2.63 g/L Mg^{2+}). The culture was spiked with acetate to reach a concentration of about 100 g/L with each feed.

Analytical Procedures

[0093] Liquid samples were taken using plastic sterile syringes and filtered through 0.20 μm syringe filter. The samples were kept in glass vials and refrigerated at 4°C before analysis. Perchlorate concentrations were determined by using a Dionex DX-500 ion chromatograph (Dionex Corp., Sunnyvale, CA) equipped with a Dionex IonPac AS16 4mm separation column, an AG16 4mm guard column, a GS50 gradient pump, an AS40 automated sampler, and a CD25 conductivity detector. An AMMS suppressor using 70 mM H_2SO_4 solution as regenerant was also used in an external cycling mode. A 1000 μL sample loop was used to detect the low perchlorate concentrations in the ion-exchange brine. The eluent concentration was 65 mM KOH prepared with ultra-pure water with 18 MΩ cm resistivity.

[0094] Nitrate, acetate and sulfate analysis were performed using a Dionex DX-100 Ion chromatograph (Dionex Corp., Sunnyvale, CA) equipped with an IonPac AS12 4mm separation column, an AG12 4mm guard column, an AS40 automated sampler, an ASRS-ULTRA suppressor (100 mA), and a 25 μL sample loop. The eluent used was a solution containing 0.3 mM $NaHCO_3$ and 2.7 mM Na_2CO_3 and the flow rate was 1.25 mL/min.

[0095] Mg^{2+}, Ca^{2+} and K^+ concentrations were determined by flame flame atomic absorption spectrometry (Perkin Elmer, AAnalyst 300) equipped with Perkin Elmer Lumina™ Lamp. Volatile suspended solids (VSS) were measured according to the procedures described in Standard Methods (APHA, 1998).

Ingredient Effects on Microbial Growth in Synthetic Brine Solutions

[0096] Later, the inventors designed experiments to determine the effects of different ingredients present in the synthetic brine solutions. The inventors identified major differences in the relative

concentrations of Mg^{2+}, Ca^{2+}, K^+ ions in the synthetic medium verses the concentrations of those ions in seawater. Several experiments were performed to determine the effects of adding these cations to brine or leaving them out of the synthetic medium. Example results are presented in Figure 2, where a 60 g/L synthetic medium was tested to determine changes in microbial activity when each of the three above-identified ions are removed from the medium. As shown in Figure 2, the results showed that leaving out Mg^{2+}, Ca^{2+}, or K^+ caused a slowing of perchlorate degradation, *i.e.*, removing each ion was detrimental to the operation of the culture to degrade perchlorate.

[0097] After reviewing these results, the inventors focused on the concentration of divalent cations and specifically on a mole ratio of divalent cations to monovalent cation (Na^+). Experiments were then directed to laboratory and pilot plant run to determine the effects of a divalent to monovalent cation mole ratio using Mg^{2+} and/or Ca^{2+} as the divalent cations. The experiments were directed to determine the divalent/monovalent cation mole ratio, as well as to determine which of these cations would allow the generation of brine solution that could support a stable culture and to determine the operating ranges of the brine solution. The inventors found that the addition of either Ca^{2+} or Mg^{2+} to adjust the divalent to monovalent cation mole ratio resulted in a brine solution capable of supporting microbial cultures, which are capable of reducing perchlorate concentration in the brine solution rapidly and completely. However, because Ca^{2+} ions precipitated out of the brine due to high levels of carbonates, we turned our attention to the addition of Mg^{2+} ions, which did not precipitate in brine solution having high concentrations of carbonates ions. The addition of either cation was beneficial: Ca^{2+} briefly improved the perchlorate destruction rate in the brine solution prior to its elimination by precipitation, whereas Mg^{2+} remained in the brine solution and improved its long-term performance for perchlorate destruction.

[0098] The inventors also found that the culture in brine solutions having a 60 g/L NaCl concentration required more Mg^{2+} than the culture in brine solutions having a 30 g/L NaCl concentration, especially when nitrate is also present in the culture. These results verify that the requirement is not for a single concentration of divalent cations such as Mg^{2+}, but for a ratio of divalent to monovalent cation mole ratio or the Mg^{2+} to Na^+ mole ratio. Currently, the inventors know that when the ratio of divalent to monovalent cation mole ratio is at or above about 0.05 as shown in Figures 2 and 3, the cultures can reduce perchlorate rapidly in brine solution having 30 or 60 g/L NaCl, and when the ratio is increased, the culture performance increases as well. This data evidenced that a minimum concentration of 600 mg/L of Mg^{2+} in a brine solution

including 30 g/L NaCl, which corresponds to a Mg^{2+}/Na^{+} mole ratio of at or above 0.05.

[0099] As a result of these experiments, the inventors have developed a novel biological perchlorate destruction process for treating ion-exchange brine so that the brine solution can be reused or disposed of as non-hazardous waste. This ion-exchange biological perchlorate destruction process eliminates perchlorate ion from waste brine solution and conserves regenerant brine solution for reuse. The inventors have also discovered one preferred biologically stable brine solution for the destruction of perchlorate contaminated brine solutions, where the brine solution has sufficient magnesium ions to produce a magnesium to sodium or divalent to monovalent cation mole ratio ≥ 0.05.

[0100] The inventors also found that Ca^{2+} ions can be added to the brine solution to adjust the divalent to monovalent cation mole ratio and achieve a biologically stable brine solution capable of microbial growth and proliferation, where the microbes are capable of decomposing perchlorate. However, Ca^{2+} is not a preferred ion, because ion-exchange brines typically contain high concentrations of carbonates ion that tend to precipitate Ca^{2+} ions. Thus, using Ca^{2+} ions as the divalent metal will require a Ca^{2+} source be added to each batch or on a continuous basis, whereas Mg^{2+} does not precipitate out, and is able to persist in the brine solution through the recycle process. However, Ca^{2+} or a mixture of Mg^{2+} and Ca^{2+} can be used in contaminated brine solutions having no or low concentrations of carbonates.

[0101] The perchlorate degradation rates from two separate experiments using cultures fed ion–exchange brine produced from a pilot plant operated by Montgomery Watson Harza in LaPuente CA, have been normalized; the highest rate in each experiment set as 1 and the others normalized to that as shown in **Figure 5**. Experiment 1 (circles) was carried out in low biomass conditions, while experiment 2 (squares) used high biomass conditions. The optimal magnesium addition in a 3% NaCl ion-exchange brine solution was about 1200 mg/L Mg^{2+}. Comparatively higher perchlorate reduction rates were found when the subcultures were amended with 300-2200 mg/L Mg^{2+}. Out of this range, however, the rate of perchlorate was reduced compared to that of the optimal magnesium amendment.

[0102] Direct demonstration of the effect of the magnesium ions on the degradation of perchlorate in ion-exchange brine solutions was demonstrated in an actual perchlorate removal system. The perchlorate concentrations in the effluents, after 22 hour react and 2 hour settle periods, during daily 50% volume replacement of SBR feeds of ion-exchange brine produced by the ion-exchange brine solution process as operated in La Puente CA were followed over a 32 day period as shown in **Figure 6**. The brine solution was amended with acetate as an electron

donor. One culture received brine that also received magnesium amendment of 2.6 g/L MgCl. The NaCl concentration in the brine was 52 g/L. Each culture was 300 mL in volume.

[0103] The results demonstrate that the addition of magnesium to the ion-exchange brine solution is necessary for long term stable microbial activity and long term perchlorate degradation.

[0104] In an analogous fashion, brine solutions contaminated with other non ion-exchangable pollutants can be treated in brine solutions having a divalent to monovalent mole ratio of at least 0.05. For example, for oil contaminated brine solutions, the brine solution is adjusted to a divalent to monovalent cation mole ratio at or above about 0.05 and inoculated with microorganisms capable of growing in the stabilized brine solution and capable of anaerobic/anoxic degradation of the oil in the oil contaminated brine solution. For example, for brine solution contaminated with other pollutants, the brine solution is adjusted into the stable regime evidence by a divalent to monovalent cation mole ratio and inoculated with microorganisms capable of growing in the stabilized brine solution and capable of anaerobic/anoxic degradation of the pollutant in the pollutant contaminated brine solution.

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[0106] All references cited herein are incorporated by reference. While this invention has been described fully and completely, it should be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described. Although the invention has been disclosed with reference to its preferred embodiments, from reading this description those of skill in the art may appreciate changes and modification that may be made which do not depart from the scope and spirit of the invention as described above and claimed hereafter.